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(54) A method for enzyme liberation from bacterial cells

(57) The use of a temperature sensitive prophage to procure the lysis of bacterial cells increases the yield of protein available by avoiding the use of harsh or vigorous physical and chemical treatments and by causing the lysis of al the cells in the culture. The lytic process is induced by raising the temperature of the culture to a point at which the prophage becomes unstable but which still allows bacterial metabolism to continue. The method can be applied not only to batch grown cells but also to cells grown in continuous culture.

does not teach T7 polymerase promoter
or T7 polymerase nor does it
teach heterologous protein

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The claims were filed later than the filing date within the period prescribed by Rule 25(1) of the Patents Rules 1982.

SPECIFICATION

A novel method f r enzyme liberation from bacterial cells

Traditional methods for the rupture of bacterial cells suffer from the disadvantages that the cells must usually be concentrated and cell breakage is often incomplete so that enzyme yield is less than that theoretically possible.

The induction of lysogenic bacteria involves less (if any) cell concentration and there is 100% cell lysis so that enzyme yield can be much higher.

The introduction of a temperature sensitive bacteriophage, e.g. λCI857 into a suitable strain of *Escherichia coli* makes it possible to bring about the induction of the bacteriophage and hence rupture of the bacterial cells by raising the temperature of incubation for a short period.

In a typical example, a lysogenic strain of *E. coli* is cultured at 30°C to the appropriate point in the growth cycle for maximum enzyme production. The cells are harvested and resuspended in growth medium, re-incubated for approximately 1 hr at 30°C and then the temperature raised to 42°C for 30 mins followed by a further period of 2–3 hrs at 30°C to allow complete lysis of the cells and enzyme liberation.

The technique will be eminently suitable for application in a continous process whereby growing the cells at sufficient density would obviate the need for the harvesting/resuspension phase and allow direct induction on a continuous basis.

CLAIMS

1. The use of a temperature sensitive prophage (or appropriate portion of the genome thereof) to procure the lysis of bacterial cells from within by raising the temperature of incubation, allowing the release of enzymes and other proteins into the suspending liquid.

2. The bacterial cells referred to in Claim 1 are grown in suitable culture medium until the most appropriate stage is reached for release of the desired enzyme or other protein.

3. The bacterial cells referred to in Claim 1 can be grown in continuous culture and being withdrawn continuously are subjected to a higher temperature for a suitable length of time to initiate lysis of the bacterial cells.

4. The bacterial cells referred to in Claim 3 as having been subjected to a higher temperature may be returned to a lower temperature to allow the development of lysis of the bacterial cells.

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